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Revisiting the taxonomy of *Daruvedia bacillata*HONG-LI HU^{1, 2}, JACQUES FOURNIER³, RAJESH JEEWON⁴,
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Abstract — *Daruvedia bacillata*, the type species of the monotypic genus *Daruvedia*, has rarely been collected or reported, but has been placed in many unrelated genera. This paper gives a description of the fungus based on studies of the type specimen, a collection by R.W.G. Dennis, and freshly collected material. The taxon is epitypified and a discussion on its systematic placement is provided.

Key words — systematics, *Dothideomycetes*

Introduction

We have been carrying out systematic and phylogenetic studies on the *Dothideomycetes* in order to obtain a natural classification system (Zhang et al. 2008a,b,c, 2009a,b,c). This study reports on *Daruvedia* Dennis, an ascomycete genus first proposed for *Sphaeria bacillata* Cooke, a fungus originally collected on decorticated rotten wood by Capron and described in the protologue as a “long-spored sunken *Sphaeria*” (Cooke 1871). This species was later referred to *Acerbia* (Sacc.) Sacc. & P. Syd., *Ceratostomella* Sacc., and *Ophioceras* Sacc.

None of these genera were deemed suitable when Dennis (1988) characterised *Daruvedia* based on a fresh collection from the Hebrides. Dennis (1988) found no ascomata in the type material of *Sphaeria bacillata*, but Cooke's habit sketch and drawings of the ascomata, ascus, and distinctive ascospore on the herbarium packet (FIG. 1) allowed Dennis to identify his new collection as identical with *Sphaeria bacillata* and to propose a new genus for it. Later, Dennis (1989) provided a more detailed account of the taxonomic history and derivation of the word *Daruvedia* but did not assign it to any family or order (Dennis 1988, 1989).

Barr (1994), who studied *Daruvedia* in her survey of North American pyrenomycetes, agreed with Dennis that a separate genus was needed for *Sphaeria bacillata*. Although she did not examine any specimens, Barr (1994) classified *Daruvedia* in the *Pleurotremataceae*, based on her belief that the asci were unitunicate and the genus shared characteristics with other genera included in that family. However, *Pleurotremataceae* sensu Barr is no longer accepted: the 9th edition of the Dictionary of the Fungi (Kirk et al. 2001) lists *Daruvedia* as *Dothideales* inc. sed., while the 10th Edition (Kirk et al. 2008) lists it as *Dothideomycetes* inc. sed. Eriksson (2006) and Lumbsch & Huhndorf (2007), who retain *Daruvedia* in the *Pleurotremataceae*, classify the family in *Ascomycota* inc. sed. and represented by just two genera, *Pleurotrema* and *Daruvedia*.

We carried out a study using fresh collections, the collection described by Dennis (1988) (designated here as epitype), and the *Sphaeria bacillata* type material to (1) provide a detailed description of this taxon, (2) clarify the taxonomic placement of *Daruvedia bacillata*, and (3) designate an epitype. We also present a preliminary description of an associated coelomycete.

Materials and methods

Fresh material was collected by Jacques Fournier at different seasons in France. The type specimen of M.C. Cooke and the collection of R.W.G. Dennis were loaned from the Royal Botanic Gardens, Kew (K), UK, for confirmation and more detailed descriptions.

The freshly collected samples were treated following the method used by Hyde et al. (2000) with modification. Dried materials were rehydrated with water first, before checking the morphological characters in water.

Single spore cultures were obtained with the modified method used by Goh (1999).

Total genomic DNA from specimens was extracted directly from ascomata by using Forensic Kits following the instructions. The genomic DNA from cultures was extracted following a protocol as outlined by Cai et al. (2005, 2006). Polymerase chain reaction (PCR) amplification products were obtained with the two pairs of primers, ITS4 and ITS5 (White et al. 1990) and LROR and LR5 for partial rDNA LSU (Vilgalys & Hester 1990).



FIG.1. Cooke's drawings of *Sphaeria bacillata* from the holotype (K).

Results

After examining the specimens, including the drawings of Cooke on the herbarium packet (FIG. 1) and those of Dennis (1988), we concur with Dennis that *Daruvedia* should be maintained as a distinct genus for *Sphaeria bacillata*.

We found that Cooke's type material lacked ascomata as Dennis (1988) had mentioned, but Dennis's material is still in good condition. Here we provide a detailed description based on Cooke's drawing, Dennis's specimen and drawing, and our recent collections from France and designate Dennis's specimen as an epitype.

Daruvedia bacillata (Cooke) Dennis, *Belarra* 2(4): 25, 1988.

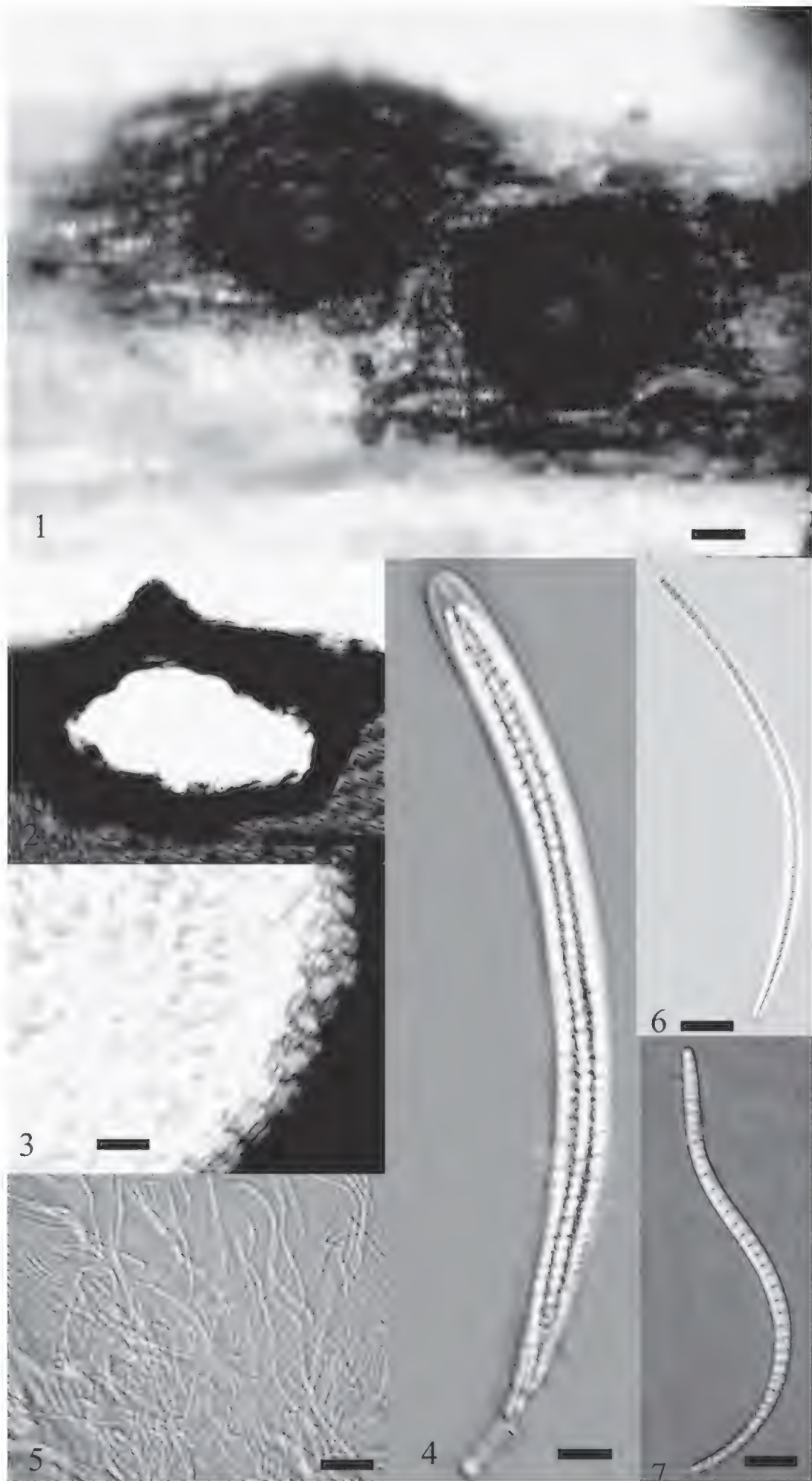
FIGS. 2–4

≡ *Sphaeria bacillata* Cooke, *Handbook of British Fungi* 2: 879, 1871.

≡ *Ophioceras bacillata* (Cooke) Sacc., *Sylloge Fungorum* 2: 360, 1883.

≡ *Ceratostomella bacillata* (Cooke) Cooke, *Grevillea* 17: 50, 1889.

≡ *Acerbia bacillata* (Cooke) Berl., *Icones Fungorum* 2: 142, 1899.



= *Rhaphidophora macrocarpa* Sacc., Nuovo Giornale Botánico Italiano 7: 306, 1875.

= *Ophioceras macrocarpum* (Sacc.) Sacc., Sylloge Fungorum 2: 359, 1883.

Ascomata scattered, rarely gregarious, erumpent through bark or wood, immersed to nearly superficial with base remaining immersed in the host tissue (FIGS. 2.1, 3.1), depressed spherical, subglobose, broadly or narrowly conical, black, roughened, 500–1000 µm high, 350–800 µm diam.; apex obtuse, pointed, discoid-flattened, up to 250 µm high, 200 µm broad (FIGS. 2.2, 3.2), sometimes hardly protruding in case of small fully immersed ascomata, and then surrounded by a black clypeus-like disc. The discs often bear tufts of brown hairs seated on an easily removed cushion-like structure; this material (a setose acervular coelomycete) was found to belong to a different taxon (see DISCUSSION) and does not represent an anamorphic state of *D. capillata* (FIGS. 3.1, 4.1). Peridium 30–40 µm thick for immersed parts, 60–100 µm thick above, two-layered; outer layer nearly homogenous, of very thick-walled cells with small lumina, inner layer textura prismatica, about 25 µm thick, of flattened cells 7–12 × 2.2–5 µm with unevenly pigmented walls, giving the appearance of alternating dark and pale columns oriented perpendicular to the surface (FIGS. 2.3, 3.3). Hamathecium of dense, very long pseudoparaphyses, 1–1.5 µm broad, sparse (FIGS. 2.5, 3.4). Asci 240–270 × 15–17 µm, 8-spored, bitunicate, but not fissitunicate, cylindrical to fusiform, short stipitate, with a narrow ocular chamber and a small inconspicuous apical apparatus (FIGS. 2.4, 3.5). Ascospores 180–200 × 4–5 µm, filiform, apex obtusely rounded without evident mucilage, base slightly tapered with inconspicuous mucilaginous material on some spores, yellowish, lying parallel in the ascus, filled with guttules, obscurely 30–40-septate, slightly constricted at septa at full maturity, smooth-walled (FIGS. 2.6–7, 3.6).

SPECIMENS EXAMINED: ENGLAND. SURREY: Shere, on dead stick, probably *Hedera*, leg. Capron 1567, M.C. Cooke (K, **holotype** of *Sphaeria bacillata*). SCOTLAND. ISLE OF ISLAY: Bridgend, Islay House, on dead stem of *Hedera helix*, old garden wall, 24 Jun. 1987, R.W.G. Dennis (K, **epitype** of *Sphaeria bacillata* **designated** here). FRANCE. Ariège, Rimont, Las Muros, on *Lonicera nigra*, 9 Jun. 1996, JF 96083; same locality, on *Acer campestre*, 17 Jun. 1996, JF 96086; same locality, on *Cornus sanguinea*, 2 Mar. 1997, JF 97057; Rimont, Le Baup, on *Populus tremula*, 14 Apr. 2005, JF 05050; same locality, on *Frangula alnus*, 14 Apr. 2005, JF 05051; Rimont, Peyrau on *Acer campestre*, 5 Oct. 2005, JF 05126; Rimont, Las Muros, on twigs of *Hedera helix*, 7 December 2005, leg. J. Fournier, det. Paul Leroy, JF 05159; same locality, on twigs of *Hedera helix*, 14 Mar. 2007, JF07026; same locality, on dead twigs of *Clematis vitalba*, 470m, 19 Jun. 2008, JF 08144; same locality, on dead decorticated twig of *Hedera helix*, 470 m, 1 Jul. 2008, JF 08155.

FIG. 2 (at left). Dennis's collection of *Daruvedia bacillata* from Scotland (K). 1. Ascomata on substrate. 2. Section of ascoma. 3. Peridium. 4. Ascus. 5. Pseudoparaphyses. 6, 7. Ascospore. Scale bars: 1, 2 = 100 µm, 3–7 = 10 µm.

Discussion

Daruvedia bacillata is uncommon but has been found on various hosts in diverse families: *Acer campestre* (Aceraceae), *Clematis vitalba* (Ranunculaceae), *Cornus sanguinea* (Cornaceae), *Frangula alnus* (Rhamnaceae), *Hedera helix* (Araliaceae), *Lonicera nigra* (Caprifoliaceae), and *Populus tremula* (Salicaceae), mainly on decorticated wood. Its occurrence is perennial. The ascoma shape and degree of immersion in the substrate are highly variable. The striking wig-like conidial hairy structure at the apex of the ascomata, which is fragile, easily removed, and often absent when fully mature, appears to be an associated fungus.

When Cooke (1871) first described this fungus, he provided a drawing of the ascomata, one ascus, and one ascospore. Later, Dennis (1988) described a new genus *Daruvedia* for this fungus, but did not assign the genus to any family or order. As *Daruvedia capillata* has bitunicate asci, it does not belong in unitunicate *Pleurotremataceae* in the broad sense of Barr (1994). Because mature fruitbodies of *D. capillata* remain embedded in sterile tissues, the genus does not belong in *Dothideales* (Kirk et al. 2001), which is characterised by the lack of hymenium when mature. Based on our results we agree with Kirk et al. (2008), who placed *Daruvedia* in the *Dothideomycetes* in the *Pleosporales*; further molecular work is needed to place the fungus in a suitable family.

We tried to isolate *Daruvedia bacillata* from single spores and conidia from the setose acervular coelomycete but failed. However, we were able to obtain pure cultures from the ascomatal spore mass. We sequenced the fungus from the cultures and were surprised when the sequence data blasted closest to *Exophiala pisciphila* McGinnis & Ajello (ITS: AF050272; LSU: DQ823101). Extraction of DNA directly from the ascomata produced the same result. As *Exophiala* spp. have teleomorphs in *Capronia* (a genus with short, fusiform, 1–2-celled ascospores), it was obvious that our isolated fungus and sequences do not derive from *Daruvedia capillata*, which has long filiform ascospores. A prolonged dry period in Pyrenees prevented our collecting more material for further study.

No anamorph has been linked to *Daruvedia bacillata*. In this study we found one associated anamorphic taxon that may represent a hyperparasite on *Daruvedia bacillata*. This unidentified fungus has acervuli comprising numerous cylindrical, long and narrow ($150\text{--}200 \times 4\text{--}5 \mu\text{m}$), thick-walled, nonseptate, brown hairs with paler obtuse ends that are often constricted just beneath the apex (FIG. 4.1) and which arise from a basal brown pseudoparenchymatous tissue with the hairs aggregating into stellate tufts (FIG. 4.2–3). Other characters include $< 2 \mu\text{m}$ diam. conidiophores that form at the base of the hairs and are composed of palisade or dense ramified, hyaline bunches (FIG. 4.4–5);, percurrent, denticulate, $< 2 \mu\text{m}$ diam. conidiogenous cells (FIG. 4.4–5), and

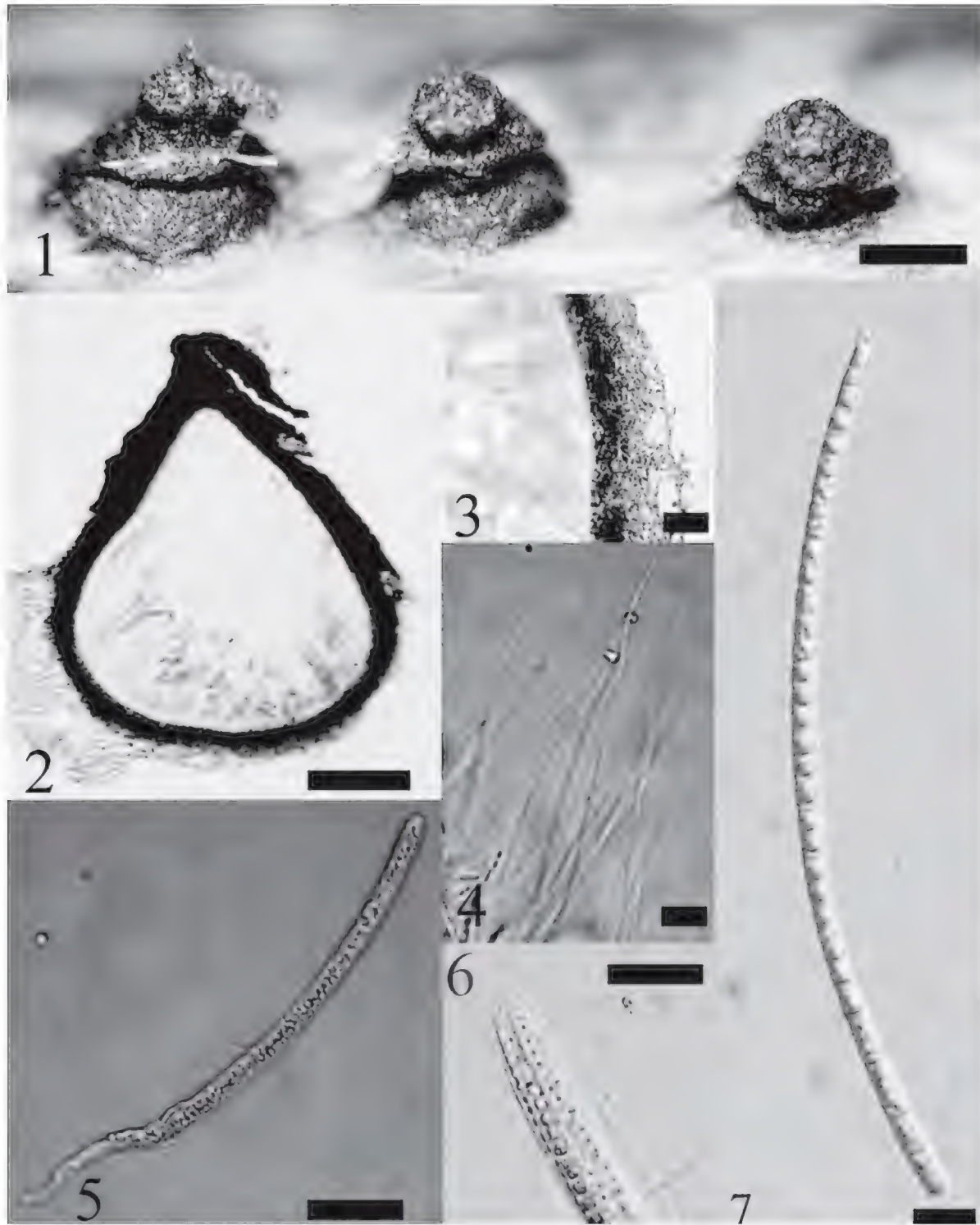


FIG. 3. Collections of *Daruvedia bacillata* from France (JF05159). 1. Mature ascoma on host surface. 2. Section of ascoma. 3. Peridium. 4. Pseudoparaphyses. 5. Ascus with ascospores. 6. Apical portion of an ascus showing ocular chamber. 7. Ascospore. Scale bars: 1 = 300 μm , 2 = 200 μm , 3 = 10 μm , 4 = 10 μm , 5 = 50 μm , 6 = 20 μm , 7 = 10 μm .

ovoid, hyaline, conidia with narrow hila and measuring $3.5\text{--}4 \times 1.8 \mu\text{m}$ (FIG. 4.5–6). This structure is only usually present on young erumpent ascomata and has always been dislodged from older mature ascomata. Further research is needed to establish the nature of the association between *D. capillata* and the unknown coelomycete.

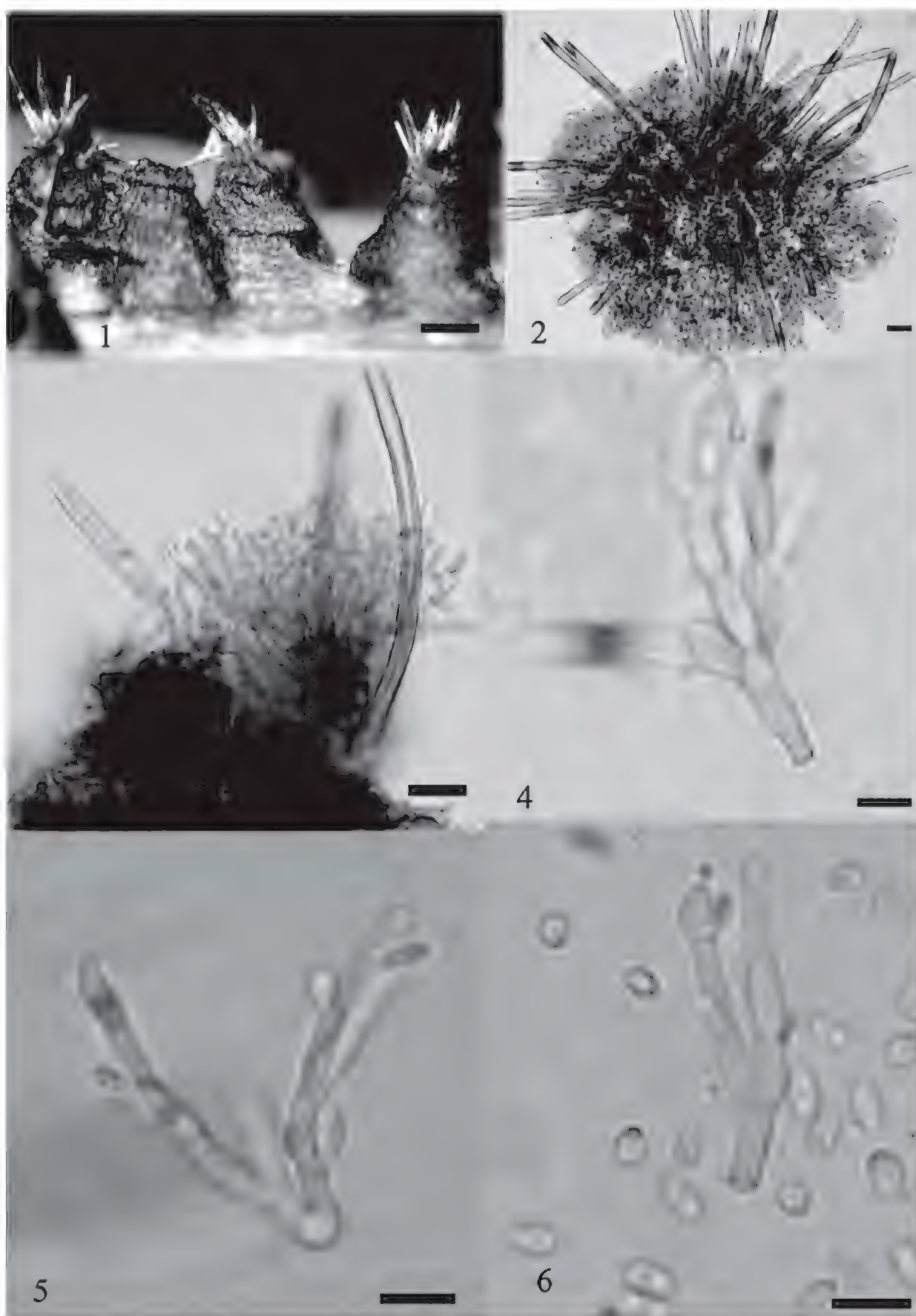


FIG. 4. Coelomycetous fungus associated with *Daruvedia bacillata*. 1. Immature *Daruvedia* ascomata with associated unknown conidiomata on natural substrate. 2, 3. Acervulus. 4–6. Conidiogenous cells and conidia from natural substrate. Scale bars: 1 = 200 μm , 2–6 = 10 μm .

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